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*Case report***A CASE OF LYMPHOCYTIC LEUKEMIA IN A BEARDED DRAGON (*POGONA VITTICEPS*) AND A REVIEW OF LITERATURE**

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The present paper reports the case of a 3 years old, female Bearded Dragon (*Pogona vitticeps*) presenting lethargy, anorexia, weight loss, and anemia and marked leukocytosis at CBC. The majority of leukocytes were lymphocytic/lymphoblastic cells (97%). Immunocytochemical staining of blood smears marked for CD3 (neg) and CD79a (pos) suggested immunophenotype B. The patient died after one month from diagnosis. Histology evidenced lymphoid infiltration in the heart, spleen, liver, kidneys and gut. In addition, in the bone marrow a massive infiltration of lymphoid cells confirmed the diagnosis of leukemia. Immunohistochemistry confirmed the CD79a positivity of a large part of infiltrating lymphoid cells indicating a B cells immunophenotype of the neoplastic population. The presence of lymphocytosis and multiorgan infiltration supported the diagnosis of lymphocytic leukemia. Finally, a revision of the literature has also been made.

Key words: Bearded dragon, *Pogona vitticeps*, leukemia, lymphocytosis, review

INTRODUCTION

The bearded dragon, *Pogona vitticeps*, is the most common reptile pet worldwide. Despite tumors of the hematological system have been documented in reptiles and in lizards, there are only occasional reports in the bearded dragon [1-4]. The prevalence of neoplasms in lizards is between 6% and 8.5% in general and of 1.6% in the case of lymphomas [3,5]. In particular, only few cases have been reported in bearded dragons: a lymphoblastic leukemia [6], a myelogenous leukemia [7], and two chronic monocytic leukemias [8,9].

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CASE PRESENTATION

A 3 years old female bearded dragon (*Pogona vitticeps*), weighting 122 grams, was visited due lethargy, anorexia, dehydration and weight loss starting the previous fortnight. The reptile was living in a pet shop and was fed with mealworms, crickets and a mixture of salads, dusted with calcium carbonate (Repti Calcium, Zoo Med Laboratories Inc., San Luis Obispo, CA USA). Due to the poor clinical presentation, supportive care was immediately started with forced feeding (Carnivore Care, Oxbow, Omaha, NE USA) and enrofloxacin (5 mg/kg, OS, SID, Baytril®, Bayer, Germany). Peripheral blood was sampled from the ventral tail vein and collected in a lithium heparin tube; CBC and biochemical profile were performed. Plasma biochemistry values were obtained with an automated clinical chemistry analyzer (Roche Hitachi 912 Chemistry Analyzer, GMI, Ramsey, MN USA). HCT was obtained with ultracentrifugation (Haematokrit 200, Hettich, Tuttlingen, Germany).

The biochemical panel showed a mild increased of uric acid (8.4 mg/dL RR 1.8-7), total proteins (6.9 g/dL RR 3.6-6.4) and globulins (3.6 g/dL RR 1.4-3.2). Anemia (HCT: 10%, RR 17-28), adequate platelets (with aggregates), and marked leukocytosis (WBC: > 100.000/μL RR 5.9-14.3) were present at CBC.

Blood smears were prepared directly during tail venipuncture. Smears were stained with May Grunwald Giemsa (MGG, Sigma-Aldrich, Saint Louis, MI USA) dye method and immediately observed under an optical microscope (BX50F4, Olympus Optical Co., LTD, Tokyo, Japan) at 20 x to evaluate cellularity and presence of platelet aggregates and at magnification 60x for differential cell count performed by evaluating 200 nucleated cells in a monolayer. A modified immunocytochemistry protocol (ICC) was applied to the 6 remaining blood smears. The antibodies and reagents used to characterize this clinical case were: anti-CD3 rat monoclonal antibody clone KT3 (Bio-Rad, Hercules, CA USA), anti-CD79a mouse monoclonal antibody clone HM57 (Abcam, Cambridge, UK), Vectastain Elite Kit which contains anti-rat mouse and anti-mouse goat IgG as a secondary antibody and avidin-biotin reagent as a tertiary reagent for signal amplification (Vector Laboratories, Inc., Burlingame, CA USA).

The majority of leukocytes were lymphocytes (95%), and rare lymphoblastic cells (2%) rare heterophils (2%) and monocytes (1%). Lymphocytic cells were of small and medium diameter, showing a high nuclear/cytoplasmic ratio and moderate blue cytoplasm and mild anisocytosis/anisokaryosis. Some cells had nuclear membrane irregularities including clives. (Fig. 1, 2). Immunocytochemical stain on blood smear marked for CD3 (neg) and CD79a (pos) suggested immunophenotype B. Despite the supportive care, the patient died one month after the diagnosis and chemotherapy was not attempted.

A necropsy was immediately performed. It evidenced severe emaciation and dehydration (Fig. 3), without other macroscopic lesions. Organs and bones were fixed in 10% buffered formalin, paraffin embedded and submitted for histological evaluation.

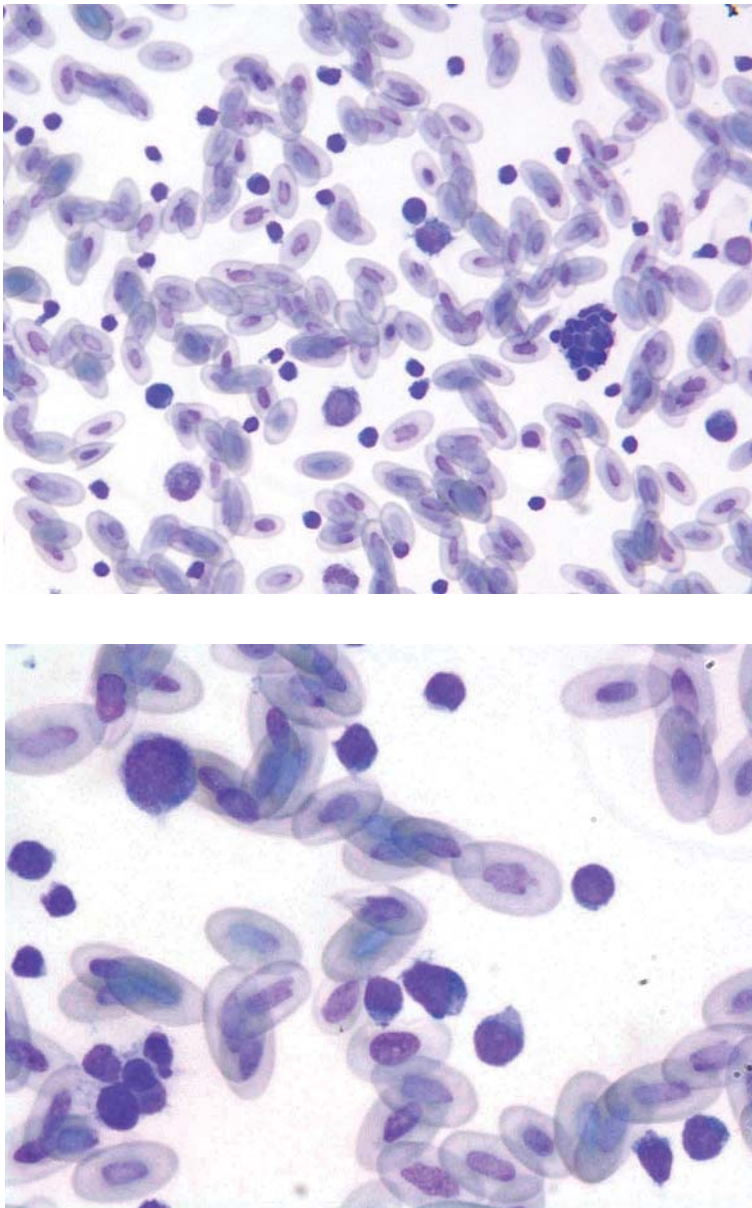


Figure 1, 2. Bearded Dragon (*Pogona vitticeps*). Blood smear 20x, 60x, MGG. Lymphocytes and rare lymphoblastic cells.

Histology, performed in serial hematoxylin and eosin (H&E) 3µm thin tissue sections, evidenced a lymphoid infiltration with a high mitotic index in the heart, spleen, liver, kidneys and gut (Fig. 4, 5). In addition, in the bone marrow, a massive infiltration of lymphoid cells and significant reduction of erythroid and megakaryocytic cell lines



Figure 3. Necropsy of the female of Bearded Dragon (*Pogona vitticeps*), with evident dehydration and weight loss.

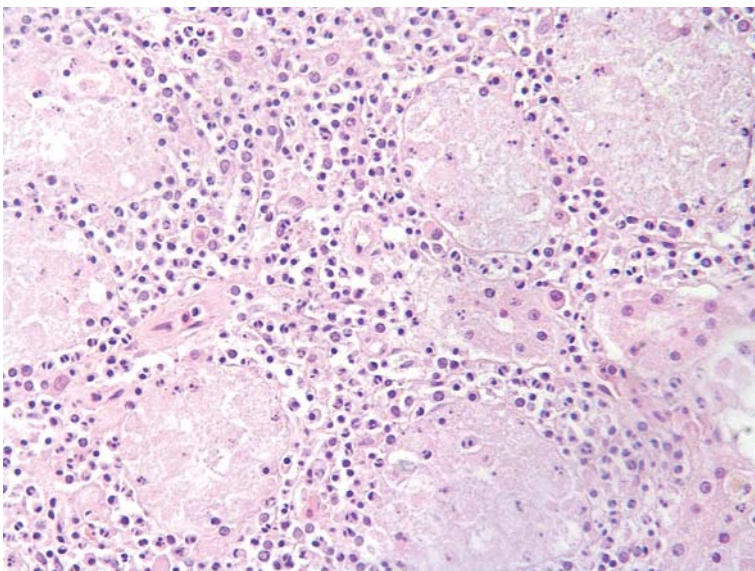


Figure 4. Bearded Dragon (*Pogona vitticeps*), kidney, 20x, H&E. Lymphoid infiltration with interstitial and periglomerular fibrosis.

confirmed the diagnosis of leukemia (Fig. 6). Immunohistochemistry performed on different organs by using the same, previously mentioned monoclonal antibodies, confirmed the CD79a positivity of a large part of infiltrating lymphoid cells indicating a B cell immunophenotype of the neoplastic population. The heart showed endocardial,

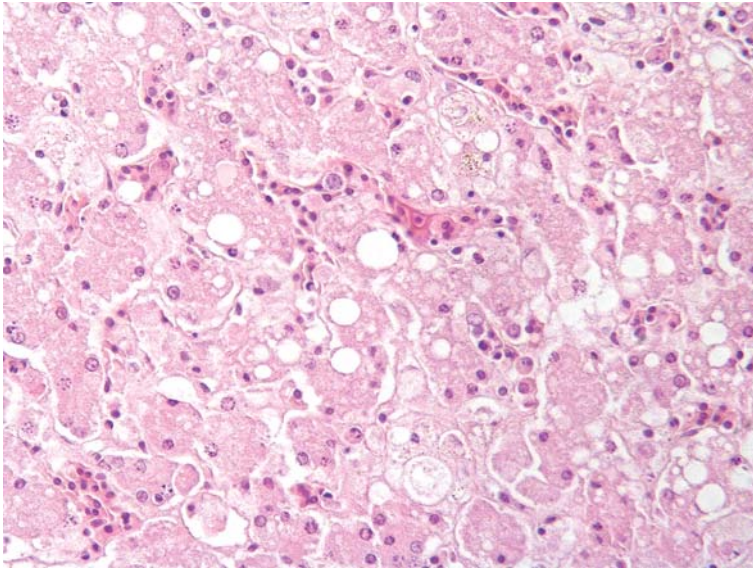


Figure 5. Bearded Dragon (*Pogona vitticeps*), liver, 20x, H&E. Lymphoid infiltration with expansion of the portal tracts, bridging infiltration and bridging fibrosis.

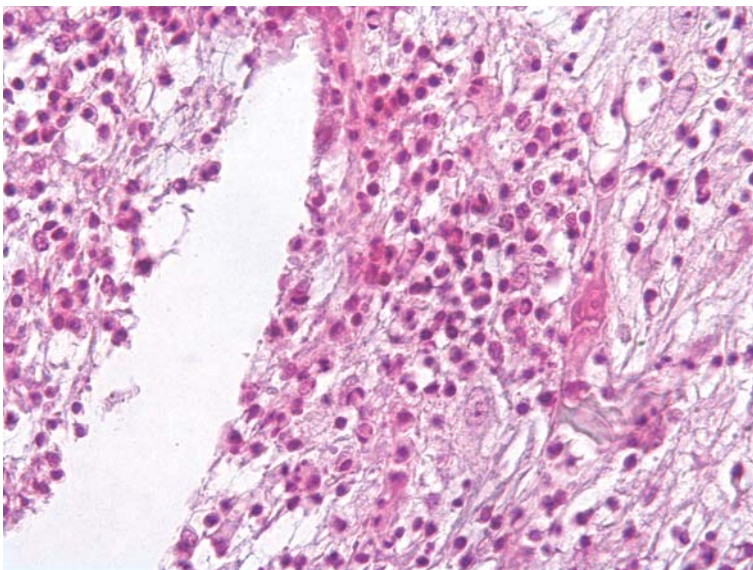


Figure 6. Bearded Dragon (*Pogona vitticeps*), bone marrow, 40x, H&E. A massive infiltration of lymphoid cells and significant reduction of erythroid and megakaryocytic cell lines

myocardial, and epicardial infiltrates associated with fibrosis, with severe endocardial fibroelastosis. The lesions observed at the level of the kidney, closely resembled the chronic renal inflammatory conditions, with interstitial and periglomerular fibrosis and tubular atrophy only in areas of leukemic infiltration, whereas no fibrosis or atrophy was observed in not infiltrated areas. The liver showed expansion of the portal tracts, bridging infiltration, bridging fibrosis, and cirrhosis with pseudo-lobule formation. In the foci of leukemic infiltration, the pancreas showed parenchymal destruction and fibrous scars, thereby resembling chronic pancreatitis. A strong association between fibrosis and the lymphocytic infiltration of chronic lymphocytic leukemia in various organs has been demonstrated. On the basis of cytological and histological/immunohistochemical findings observed in our case, a diagnosis of chronic lymphocytic leukemia, B cell immunophenotype, was made.

DISCUSSION

Neoplastic diseases have been rarely described in *Pogona vitticeps*. In a recent study on the prevalence of common disorders in 529 captive bearded dragons, that were presented to three exotic animal clinics in Central Europe in a period of three years, only seven cases of neoplasia were observed, and precisely four cases on skin neoplasia, one ganglioneuroma, one case of leukemia (female, 4 years of age) and one case of leukemia and lymphoma in kidneys, lungs and liver (male, 4 years of age) [3]. In addition, a case report of lymphoblastic leukemia in *Pogona vitticeps* was described but no clinical signs were noted in this Inland bearded dragon. No biochemical and hematological profiles were performed, but numerous intravascular neoplastic “blastic” lymphocytes were found within all visceral organs examined except the lens, esophagus and skin [6]. Also, a case of chronic monocytic leukemia was described in a *Pogona vitticeps* that had a history of lethargy, anorexia, and dehydration. Hematologic and biochemical data indicated a nonregenerative anemia and a severe leukocytosis with monocytosis. Peripheral blood smears were evaluated with cytochemical stains and only alphanaphthyl acetate (nonspecific) esterase activity was positive in the monocytoïd cells. Liver biopsies contained clusters of hepatocytes separated by severe confluent infiltrates of monocytoïd cells that replaced the normal hepatic architecture [8]. Another case report about *Pogona vitticeps* described a presumptive chronic monocytic leukemia where CBC showed a severe leukocytosis with marked monocytosis (243.5×10^3 cells/l). Immunohistochemistry for CD3 but not for CD 79 cell markers was performed and resulted negative in the neoplastic cell population. In this case, a chemotherapy treatment with cytosine arabinoside (100 mg/m² over 48 hr i.v.) was initiated but 45 hours into the treatment the dragon became acutely unresponsive and died [9]. A further report described the first known case of myelogenous leukemia in a bearded dragon. Interestingly, whole body radiographs revealed bone lysis in the proximal, midshaft, and distal right humerus and soft tissue swelling of the right elbow and stifle joints. On the blood smear the majority of leukocytes had abnormal

mononuclear lymphoid-type cells that did not stain with the lymphoid cytochemical stains a-naphthyl butyrate esterase, CD3 and CD79a. Histopathologic examination revealed an infiltration of multiple tissues, including the spleen, liver, kidney, pancreas, testes, heart, lung, brain, adipose, skeletal muscle, and skin, by sheets of neoplastic round cells [7].

Generally, all cases that have been reported (except Suedmeyer WK, 1996), showed a history of lethargy, anorexia and weight loss and in one case bone lysis was observed. In all reports, where the hematological profile was performed, anemia and marked leukocytosis were present. If carried out, the histopathologic examination revealed infiltration of multiple organs as kidney, liver, spleen and bone marrow [6-9].

Leukemias are commonly graded as acute or chronic depending on the number of blast cells in peripheral circulation, precisely, in acute leukemia, blast cells predominate whereas mature forms predominate in chronic diseases. Chronic lymphocytic leukemia is a neoplastic clonal proliferation of small, mature lymphocytes manifesting as a persistent and marked peripheral lymphocytosis [10]. In reptile lymphoid neoplasia (lymphoma and leukemia) cases, where cell-type characterization has been pursued, the majority of neoplasms were of T-cell lineage [1,11].

In our report, a few of biochemical abnormalities were identified, i.e. a mild increased of uric acid and hyperproteinemia that can correlate with the dehydration of the leukemic bearded dragon. The presence of marked peripheral lymphocytosis and multiorgan massive infiltration supported the diagnosis of lymphocytic leukemia. The CD79a positivity confirmed B-cell immunophenotype of neoplastic cells, differently than described in the literature on reptiles, where the T-cell type is prevalent [1,11]. It would be interesting to verify, on a greater number of animals, if the survival time of patients with chronic leukemia is longer than in reptiles with the acute form and if the time is related with immunophenotype, similar to what happens in mammals.

Etichal statement

All procedures have been executed in accordance with the Regulations on Animal Welfare in Veterinary Practice.

Authors' contributions

AG, VC, AC, CG, PR, and MC actively participated in clinical and diagnostic procedures of this clinical case. LG and GR actively participated in necropsy and histopathological investigation. AG, LG, MC and GR participated in writing this manuscript. All authors have approved the final version of the manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of Informed Consent:

The owner understood clinical and pathological procedure and agrees that results related to investigation of its animal could be published in this journal.

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PRIKAZ SLUČAJA LIMFOCITNE LEUKEMIJE KOD BRADATOG ZMAJA (*POGONA VITTICEPS*)

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Rad predstavlja prikaz slučaja 3 god stare ženke bradatog zmaja (*Pogona vitticeps*) sa kliničkim znacima letargije, anoreksije, gubitka težine, anemije i izražene leukocitoze. Većinom su bile prisutne limfocitne/limfoblastične ćelije (97%). Imunohistotohemi-

jskim bojenjem krvnih razmaza obeleženih na prisustvo CD3 (neg) i CD79a (poz) navode na dominantno prisustvo imunofenotipa B. Pacijent je uginuo mesec dana nakon postavljanja dijagnoze. Histološka ispitivanja su dokazala prisustvo limfoblastne infiltracije u srcu, slezini, bubregu, crevima. Pored toga, masivna infiltracija limfoblastima u koštanoj srži je potvrdila dijagnozu leukemije. Imunohistohemija je potvrdila da je većina limfoidnih ćelija CD79a pozitivna, čime se potvrđuje B imunofenotip neoplastične populacije limfocita. Prisustvo limfocitoze i limfoidne infiltracije brojnih organa potvrđuje dijagnozu limfoidne leukemije.